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Body Surface Area Prediction in *Odorrana grahami*

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Abstract Body surface area (BSA) was regarded as a more readily quantifiable parameter relative to body mass in the normalization of comparative biochemistry and physiology. The BSA prediction has attracted unceasing research back more than a century on animals, especially on humans and rats. Few studies in this area for anurans were reported, and the equation for body surface area (S) and body mass (W): $S = 9.9\ W^{0.56}$, which was concluded from toads of four species in 1969, was generally adopted to estimate the body surface areas for anurans until recent years. However, this equation was not applicable to *Odorrana grahami*. The relationship between body surface area and body mass for this species was established as: $S = 15.4\ W^{0.579}$. Our current results suggest estimation equations should be used cautiously across different species and body surface area predictions on more species need to be conducted.

Keywords body mass, body surface area, estimation equation, *Odorrana grahami*

Body surface area (BSA) was regarded as a more readily quantifiable parameter relative to body mass in the normalization of comparative biochemistry and physiology. The BSA prediction has attracted unceasing research back more than a century on animals, especially on humans and rats. The well know equation for estimating BSA was the Meeh equation, which related BSA (S) to the two-thirds power of body mass (W): $S = k\ W^{0.667}$, in which k, known as the Meeh constant, was a constant supposedly different for each species of animal. The Meeh constant for anurans has been reported as various values from 4.62 to as high as 10.6 with the exponent 0.667 since then. However, the exponent 0.667, known as the two-thirds rule, which was originally derived from homoiothermic vertebrates, may not be precise for ectothermic vertebrates such as amphibians, therefore, this exponent value was substituted later by several authors with various Meeh constants. Hutchison *et al*. (1968) gave an equation for anurans: $S = 1.131\ W^{0.579}$, which was concluded from 13 species belonging to four families of anurans. The Meeh constant of 1.131 was an abnormally small Meeh constant. Carefully checking Figure 18 on page 80 of this reference paper indicated there were obvious typing errors, the calculated values were much smaller (about ten times) than the values shown in this figure, although the accurately adjusted values could not be given here due to the original measured data being absent. Furthermore, among the equations for individual species given by them, several equations could not be merged to a single equation, for example, the predicted BSA between equations derived from *Hyla arborea* ($S = 0.905\ W^{0.823}$) and from *H. cinerea* ($S = 1.462\ W^{0.273}$) was significantly different from the results of ANOVA (data not shown). McClanahan and Baldwin (1969) gave an equation: $S = 9.9\ W^{0.56}$ (1), which was concluded from toads of four species. The study on BSA prediction in anurans was hardly reported from then, and the equation (1) was generally adopted to estimate BSA for more species of anurans until recent years.

The requirement for more precise BSA prediction increased with further study in comparative biochemistry and physiology in skin secretions of amphibians. Abundant active molecules, especially antimicrobial peptides, have been found in the skin secretions of *Odorrana grahami* (Boulenger, 1917) (Anura: Ranidae)

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from two locations: Mianning, Sichuan province and Kunming, Yunnan province, China, implying this species was a good research object to compare the skin secretion of amphibians with geographical interval. The isolation, characterization and quantification of skin secretions of *O. grahami* from different sites and seasons were currently ongoing in our laboratory. The estimation of the BSA for *O. grahami* was a necessary and related work; however, we found the generally adopted equation (1) was not applicable to this species (see below). Here, we report the relationship, expressed by the equation, between body mass and BSA for *O. grahami* for the first time.

Animals used for BSA measurement were captured in a stream approximately two meters in width located in Mianning, Sichuan province on 25 July and 18 September 2010 (E 102° 9.175', N 28° 32.833', altitude 1804 m). The animals were anesthetized with chlorobutanol. Body masses were recorded and the male and female adults were distinguished by the secondary sex characters of males: forelimb distinctly thickened, the inner dorsal side of the base of the first finger with a strong nuptial pad, dorsal sides of body with lineae musculinae. Individuals, the size smaller than the minimum male, difficult to sex were designated as subadults. A wrap method was used to measure the BSA (Jaworski and Holm, 1992). The protective paper adhered on the Parafilm® (Cat No. 701605) was cut to thin strips or pieces, and then were stuck with the reserved side on the skin. The approximate BSA was calculated by the equation:

BSA (cm^2) = area of total paper (cm^2) − area of left paper (cm^2).

The average of two measurements was used for analysis. The animal protocols were approved by the Animal Care and Use Committee of the Chengdu Institute of Biology, Chinese Academy of Sciences.

All statistical analyses were run in SPSS® Statistics (v 21.0, IBM-SPSS Inc., Chicago, Il, USA) unless specified. Where appropriate, normality was tested using Kolmogorov-Smirnov's and homogeneity using Levene's tests. In consideration of sexual dimorphism, one-way analysis of covariance (ANCOVA) was used to compare slopes and intercepts of regression lines for the males, females and subadults performed by GraphPad Prism® (version 5.0, GraphPad Software, San Diego, CA, USA). If neither the slopes nor the intercepts differed, a common regression equation could be given. The BSAs measured in July were used for linear regression of $\log_{10} S$ on $\log_{10} W$ by the method of two-stage least-squares. The BSAs measured in September were used to further verify the validity for the derived equation and the empirically predictive equation (1) by using a one-way analysis of variance (ANOVA) and Tukey's post hoc test. The accuracy and robustness of the predictive function by regressing measured BSA on predicted BSA were tested.

The measured body mass and BSA in July (n = 73, 8 subadults, 39 females, 26 males) are shown in Table 1. The results of ANCOVA indicated that the three regression lines were identical among males, females and subadults, since the differences among these slops ($F_{2, 67} = 0.132$, $P = 0.877$) and intercepts ($F_{2, 69} = 0.894$, $P = 0.414$) were not significant (Figure 1). Thus, the BSA of *O. grahami* relative to body mass was concluded to be the common equation: $\log_{10} S = 1.187 + 0.579 \log_{10} W$ (2) ($F_{1, 71} = 386.197$, $r^2 = 0.843$, $P < 0.001$) with the 95% confidence interval for the slope 0.520 – 0.638. The mean absolute relative error (%) with one standard error (1SE)

Table 1 BSA estimated by equation (1) and (3).

Body mass (g)		Measured BSA (cm^2)		Predicted BSA by equation (1) (cm^2)		ARE for equation (1) (%)		Predicted BSA by equation (3) (cm^2)		ARE for equation (3) (%)		Number of animals	Sex
Mean	1SD	Mean	1SD	Mean	1SD	Mean	1SE	Mean	1SD	Mean	1SE		
Collected on 25 July, 2010:													
13.4	2.8	67.32	10.59	42.05	5.04	37.0	2.0	68.72	8.51	7.8	1.7	8	subadult
35.8	16.4	121.53	33.18	71.75	17.67	40.1	1.3	119.48	30.48	10.6	1.4	39	female
23.2	5.6	93.27	13.37	57.18	7.35	38.3	1.1	94.42	12.57	5.9	1.4	26	male
28.8	14.8	105.52	31.77	63.30	16.98	39.1	0.8	104.99	29.2	8.6	0.9	73	Total
Collected on 18 September, 2010:													
28.7	13.7	114.98	33.88	63.6	15.64	43.4	2.5	105.47	26.91	10.7	2.8	10	Total

Note: 1SD: one standard deviation; 1SE: one standard error; ARE: absolute relative error; equation (1): $S = 9.9 W^{0.56}$; equation (3): $S = 15.4 W^{0.579}$.

between the measured BSA and predicted BSA was 7.8 (1.7) for subadults, 10.6 (1.4) for females, 5.9 (1.4) for males and 8.6 (0.9) for total (Table 1).

Equation (2) was transformed to equation: $S = 15.4\ W^{0.579}$ (3). The predicted BSA was calculated by using the equation (1) and (3) respectively, based on the corresponding measured body mass in September (n = 10) shown in Table 1. Differences among the measured BSA and the predicted BSA was significant by ANOVA ($F_{2,27} = 10.593$, $P < 0.001$), Tukey's post hoc comparisons indicated that the measured BSA was significantly different from predicted BSA by equation (1) ($P < 0.001$), whereas was not significantly different from predicted BSA by the equation (3) ($P = 0.706$), hence, the equation (1) with mean absolute relative error (%) 43.4 (1SE: 2.5) was not applicable to the BSA prediction for *O. grahami* (Table 1). The regression line describing the relationship between measured BSA and predicted BSA calculated by equation (3) was significant (measured BSA = 1.156 × predicted BSA – 6.980; $F_{1,8} = 43.138$, $r^2 = 0.824$, $P < 0.001$) with the 95 % confidence interval for the slope 0.750–1.562. The regression parameters (e.g. slope approach to 1, intercept approach to 0 and high r^2) indicate that the predicted BSA was approximate to the measured BSA with mean absolute relative error (%) 10.7 (1SE: 2.8) (Figure 2, Table 1), hence, the equation (3) was applicable to the BSA prediction for *O. grahami*.

The exponent 0.579 of the present reported equation (3) was the same as that given by Hutchison *et al*. (1968). We speculated they reported the $\log_{10} k$ as k. Thus, the present estimation equation for *O. graham* would approximate to the adjusted equation given by them. The equation (1) was concluded merely from anurans of four species: *Scaphiopus couchi*, *Bufo punctatus*, *B. boreas* and *B. cognatus*, although it has been confirmed to be a good estimation of BSA for *Litoria caerulea* and *L. chloris* (Buttemer, 1990). These toads were much stouter than the *O. grahami*, therefore, it is reasonable that the estimation BSA by the equation (1) concluded from the toads was different with the equation (3) concluded from *O. grahami* (Table 1). Our current results suggested the estimation equations for BSA should be used cautiously in different species and the BSA predictions on more species needed to be conducted.

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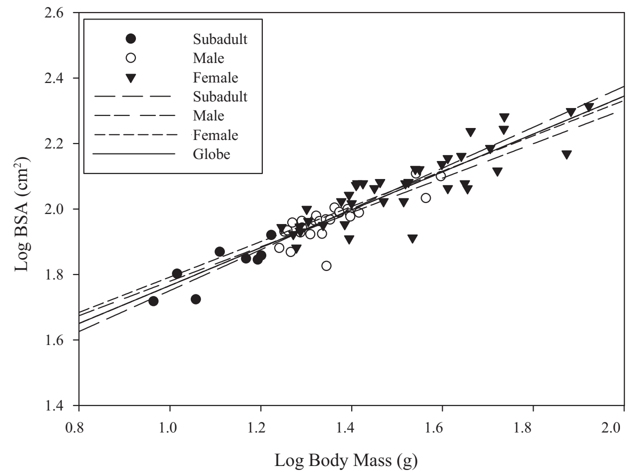

Figure 1 The relationship between body mass and BSA based on the measured data collected in July 2010 (n = 73, 8 subadults, 39 females, 26 males).

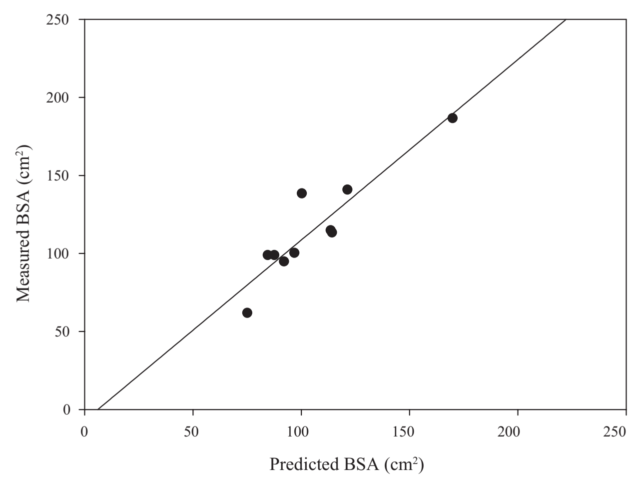

Figure 2 The regression line describing the relationship between the measured BSA collected in September 2010 (n=10) and predicted BSA calculated by equation (3).

References

Buttemer, W.A. 1990. Effect of temperature on evaporative water loss of the australian tree frogs *Litoria caerulea* and *Litoria chloris*. Physiol Zool, 63: 1043–1057

Hutchison, V.H., Whitford, W.G., Kohl, M. 1968. Relation of body size and surface area to gas exchange in anurans. Physiol Zool, 41: 65–85

Jaworski, A., Holm, J.C. 1992. Distribution and structure of the population of sea lice, *Lepeophtheirus salmonis* Krøyer, on Atlantic salmon, *Salmo salar* L., under typical rearing conditions. Aquac Res, 23: 577–589

McClanahan, J., Lon, Baldwin, R. 1969. Rate of water uptake through the integument of the desert toad, *Bufo punctatus*. Comp Biochem Physiol, 28: 381–389

Supplemental Materials

Supplemental S1 BSA of individuals estimated by equation (1) and (3).

Body mass (g)	Measured BSA (cm^2)	Predicted BSA by equation (1) (cm^2)	ARE for equation (1) (%)	Predicted BSA by equation (3) (cm^2)	ARE for equation (3) (%)	Sex
Collected on 25 July, 2010:						
9.2	52.27	34.30	34.4	55.66	6.5	&
10.4	63.44	36.74	42.1	59.76	5.8	&
11.4	52.98	38.68	27.0	63.02	18.9	&
12.9	74.00	41.45	44.0	67.69	8.5	&
14.7	70.56	44.60	36.8	73.01	3.5	&
15.6	70.04	46.11	34.2	75.57	7.9	&
15.9	72.07	46.60	35.3	76.41	6.0	&
16.7	83.23	47.90	42.4	78.61	5.6	&
17.4	75.99	49.02	35.5	80.50	5.9	♂
17.6	88.00	49.33	43.9	81.03	7.9	♀
17.9	85.26	49.80	41.6	81.83	4.0	♂
18.1	86.00	50.11	41.7	82.36	4.2	♂
18.4	73.91	50.57	31.6	83.15	12.5	♂
18.6	90.91	50.88	44.0	83.67	8.0	♂
18.7	83.74	51.03	39.1	83.93	0.2	♀
19.0	76.13	51.49	32.4	84.71	11.3	♀
19.3	88.31	51.95	41.2	85.48	3.2	♀
19.4	84.73	52.10	38.5	85.73	1.2	♂
19.4	85.26	52.10	38.9	85.73	0.6	♀
19.5	87.80	52.25	40.5	85.99	2.1	♂
19.5	92.00	52.25	43.2	85.99	6.5	♂
20.0	99.98	52.99	47.0	87.26	12.7	♀
20.2	91.87	53.29	42.0	87.76	4.5	♀
20.4	83.74	53.58	36.0	88.27	5.4	♂
20.8	92.37	54.17	41.4	89.26	3.4	♂
21.0	95.41	54.46	42.9	89.76	5.9	♂
21.3	91.86	54.89	40.2	90.50	1.5	♂
21.6	84.00	55.33	34.1	91.24	8.6	♂
21.7	89.32	55.47	37.9	91.48	2.4	♀
22.0	93.00	55.90	39.9	92.21	0.8	♂
22.1	66.99	56.04	16.3	92.45	38	♂
22.5	92.87	56.61	39.0	93.42	0.6	♂
23.0	101.00	57.31	43.3	94.62	6.3	♂
23.6	97.50	58.14	40.4	96.04	1.5	♂
23.6	98.00	58.14	40.7	96.04	2.0	♂
23.8	105.56	58.41	44.7	96.51	8.6	♀
24.2	89.83	58.96	34.4	97.44	8.5	♀
24.6	100.01	59.51	40.5	98.37	1.6	♂
24.8	81.20	59.78	26.4	98.83	21.7	♀
24.8	110.40	59.78	45.9	98.83	10.5	♀
24.9	94.90	59.91	36.9	99.06	4.4	♂
24.9	96.93	59.91	38.2	99.06	2.2	♂
25.1	104.08	60.18	42.2	99.52	4.4	♀
25.7	118.25	60.98	48.4	100.90	14.7	♀
25.9	119.77	61.25	48.9	101.35	15.4	♀
26.1	97.50	61.51	36.9	101.80	4.4	♂
26.6	119.77	62.17	48.1	102.93	14.1	♀
28.2	115.71	64.24	44.5	106.47	8.0	♀
29.0	120.80	65.25	46.0	108.21	10.4	♀
29.5	105.56	65.88	37.6	109.28	3.5	♀
32.7	105.56	69.79	33.9	116.00	9.9	♀

Continued Supplemental S1

Body mass (g)	Measured BSA (cm^2)	Predicted BSA by equation (1) (cm^2)	ARE for equation (1) (%)	Predicted BSA by equation (3) (cm^2)	ARE for equation (3) (%)	Sex
33.0	119.77	70.15	41.4	116.61	2.6	♀
33.5	120.91	70.74	41.5	117.63	2.7	♀
34.2	81.71	71.56	12.4	119.05	45.7	♀
34.7	132.46	72.15	45.5	120.05	9.4	♀
34.8	128.31	72.26	43.7	120.25	6.3	♂
35.4	132.18	72.96	44.8	121.45	8.1	♀
36.6	108.00	74.33	31.2	123.82	14.6	♂
39.5	126.00	77.58	38.4	129.40	2.7	♂
39.7	137.03	77.80	43.2	129.78	5.3	♀
40.9	115.71	79.10	31.6	132.04	14.1	♀
40.9	142.98	79.10	44.7	132.04	7.7	♀
43.8	145.15	82.20	43.4	137.38	5.4	♀
44.6	119.77	83.04	30.7	138.83	15.9	♀
45.2	115.61	83.66	27.6	139.91	21.0	♀
45.9	172.87	84.38	51.2	141.16	18.3	♀
50.7	153.47	89.22	41.9	149.53	2.6	♀
52.6	130.94	91.07	30.4	152.75	16.7	♀
54.3	175.60	92.71	47.2	155.59	11.4	♀
54.5	191.59	92.90	51.5	155.92	18.6	♀
74.7	147.68	110.84	24.9	187.14	26.7	♀
76.4	198.94	112.25	43.6	189.60	4.7	♀
83.6	206.07	118.05	42.7	199.75	3.1	♀
Collected on 18 September, 2010:						
15.4	61.92	45.78	26.1	75.01	21.1	&
18.9	98.96	51.34	48.1	84.45	14.7	♀
20.1	98.96	53.14	46.3	87.51	11.6	♀
21.9	94.90	55.76	41.2	91.97	3.1	♂
23.9	100.38	58.55	41.7	96.74	3.6	♂
25.4	138.55	60.58	56.3	100.21	27.7	♀
31.5	114.90	68.34	40.5	113.51	1.2	♂
31.8	113.50	68.71	39.5	114.14	0.6	♂
35.3	140.92	72.84	48.3	121.25	14.0	♀
63.2	186.76	100.93	46.0	169.88	9.0	♀

Note: &: subadult, ♂: male, ♀: female, ARE: absolute relative error.

Supplemental S2 References in which the equation (1) was adopted to estimate BSAs for some species of anurans without verifying the validation [1–3],

[1] **Christian K., Parry D.** 1997. Reduced rates of water loss and chemical properties of skin secretions of the frogs *Litoria caerulea* and *Cyclorana australis*. Aust J Zool, 45:13–20

[2] **Gammill W. M., Scott F. J, Rollins-Smith L. A.** 2012. Norepinephrine depletion of antimicrobial peptides from the skin glands of *Xenopus laevis*. Dev Comp Immunol, 37: 19–27

[3] **Withers P. C., Hillman S. S.** 1982. Water loss and nitrogen excretion in sharp-nosed reed frogs (*Hyperolius nasutus*: Anura, Hyperoliidae). J Exp Biol, 97: 335–43

Supplemental S3 Introduction and parameters for comparison of several regression lines by ANCOVA.

Zar [4] gives a method for comparing the slopes and intercepts of regression lines using analysis of covariance (ANCOVA). This analysis is available as a module for linear regression in GraphPad Prism®, and was widely used [5–7]. The starting point is to compare the slopes using an *F*-test. If the slopes are not significantly different (i.e., $P > 0.05$) then it is possible to use a further *F*-test to compare the intercepts to decide whether the lines are identical. If *P* for this comparison is > 0.05, then one can conclude that the lines are identical.

In the present research, the parameter for ANCOVA was set as following:

1. Comparing the slopes

Dependent variable: $\log_{10} S$

Fixed factor: group (subadult, male and female)

Covariate: $\log_{10} W$

Model (input in the order): x, group, x*group

Sum of squares: TypeI

Result: P of x*group > 0.05

2. Comparing the intercepts

Same as 1 except the x*group was removed from the Model, i.e., the interaction between the covariate and fixed factor was not considered in this step.

Result: P of group > 0.05

[4] **Zar J. H.** 2009. Comparing Simple Linear Regression Equations Biostatistical analysis. (5th ed.) Prentice-Hall, Chapter 18, 372–375

[5] **Kotronen A., Juurinen L., Tiikkainen M., Vehkavaara S., Yki-Järvinen H.** 2008. Increased liver fat, impaired insulin clearance, and hepatic and adipose tissue insulin resistance in type 2 diabetes. Gastroenterology, 135: 122–130

[6] **Tommasini S. M., Nasser P., Jepsen K. J.** 2007. Sexual dimorphism affects tibia size and shape but not tissue-level mechanical properties. Bone, 40: 498–505

[7] **Battino D., Croci D., Rossini A., Messina S., Mamoli D., Perucca E.** 2003. Serum carbamazepine concentrations in elderly patients: A case matched pharmacokinetic evaluation based on therapeutic drug monitoring data. Epilepsia, 44: 923–929